18.4.3.2 The observed signal; calibration function

The *calibration function* is defined as the functional (not statistical) relationship for the CMP, relating the expected value of the observed signal or response variable $E(y)$ to the analyte amount x. The corresponding graphical display for a single analyte is referred to as the calibration curve. When extended to additional variables or analytes which occur in multicomponent analysis, the "curve" becomes a calibration surface or hypersurface. The functional relationship, $E(y) = F(x)$, may in general be quite complicated -- and functions assumed (for data reduction) may be wrong, thus comprising a source of systematic error. We consider here only the simplest case, the linear calibration curve, where the *observed signal* or *response* y is given by

$$y = F(x) + e_y \tag{18.4.1}$$

with

$$F(x) = B + S = B + Ax \tag{18.4.2}$$

where S denotes the *net signal*; B, the *blank* (or *background* or *baseline*, as appropriate); x, the analyte amount or concentration; and A, the *sensitivity*. The error e_y is taken to be random and normal, with zero mean (no bias) and dispersion parameter σ (standard deviation). The estimated net signal is thus

$$\hat{S} = y - \hat{B} \tag{18.4.3}$$

In the more general case of multicomponent analysis Eq. (1) takes the form

$$\boldsymbol{y} = \boldsymbol{F(x)} + \boldsymbol{e}_y \tag{18.4.4}$$

where $\boldsymbol{y}$, $\boldsymbol{x}$, and $\boldsymbol{e}_y$ are vectors, and the Calibration Function takes into account the response relations for all analytes and interferences. Under the best of circumstances, Eq. (4) is a linear matrix equation.

Notes:

(1) Symbols used to represent the calibration parameters vary among disciplines. In statistics, for example, it is conventional to use β_i -- e.g., for a quadratic relationship: $F(x) = \beta_o + \beta_1 x + \beta_2 x^2$. In analytical chemistry, identification of β_o and β_1 with the blank B and sensitivity A, respectively, is valid *only* if the calibration data represent the *entire* CMP and the calibration relation is linear.

(2) The symbol B appearing in Fig. 18.4.1, and in Equations 18.4.2 et seq., refers to the blank, as manifest in the sample itself - i.e., as impacted by matrix and interference effects, and multiple occurrences (injection points) and partial recoveries -including possible effects of the sample configuration and composition on the instrumental background. Inadequate attention to this

matter, either through properly designed "blank" experiments or thorugh appropriate adjustments for differing sensitivities, will invalidate the use of the additive model and lead to biased analyte concentration estimates. The reader is encouraged to examine the discussions in the following papers:

K. Camman: Z. Anal. Chem. 312 (1982) 515-516;
L.A. Currie: Detection in Analytical Chemistry Ch.1, ACS Symp. Series 361, Am. Chem. Soc, Washington (1988).

Sensitivity

In metrology and in analytical chemistry, the sensitivity A is defined as the slope of the calibration curve. (If the curve is in fact a "curve", rather than a straight line, then of course sensitivity will be a function of analyte concentration or amount.) If sensitivity is to be a unique performance characteristic, it must depend only on the CMP, not upon scale factors. For this reason the slope dy/dx must be defined in absolute terms, such as $mV/\mu g$.

Notes:

(1) Alternative uses for this term in analytical chemistry, such as a qualitative descriptor for *detection* capability, or slope A divided by σ, etc., are not recommended.

(2) It is recognized that the term "sensitivity" has different meanings for different disciplines. In clinical chemistry (diagnostics), for example, sensitivity is defined as "the fraction of all affected subjects in whom the test result is positive: best positivity in the presence of the disease".

(3) When a measurement process parameter is estimated by performing an operation on the observed responses, the resulting statistic is called an *estimator*; it is designated by a circumflex as shown in equations 18.4.3 and 18.4.5. Thus, $\hat{A}$ indicates an estimator such as the least squares estimator for the sensitivity, and its standard deviation $\sigma(\hat{A})$ determines the random uncertainty component for any particular estimate. If $E(\hat{A})$, the *expectation* or mean value of the $\hat{A}$ distribution, equals the true value A, the estimator is said to be unbiased.